Response of microbial respiratory electron transport activity to particulate organic matter features in the Ross Sea

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Abstract

Microbial respiration was studied measuring the ETS activity in three areas of the Ross Sea during summer 2014, in the framework of the Ross Sea Mesoscale Experiment (ROME) project. At the same time, sampling for particulate organic matter (POM) was carried out. The ETS activity rates were similar to those previously observed in other temperate and polar environments. In the epipelagic layer (0-200 m) the ETS activity showed a macroscale variability between the three sampling areas, with the lowest values at the northernmost, offshore site (hereafter called ROME1) (0.20±0.02 µl O_2 l^{-1} h^{-1}) and the highest in the 0-30 m layer of the southernmost and offshore site (ROME 3) (0.61±0.18 µl O_2 l^{-1} h^{-1}). ROME 2, placed coastward next to the Terra Nova Bay winter polynya, showed the highest variability (0.49±0.19 µl O_2 l^{-1} h^{-1}). Significantly higher values at some discrete depths were measured, depending on the proximity to the frontal zone that crossed the area. Different hydrological features (upper mixed layer depth, current intensity, fronts and a cyclonic eddy) as well as residual ice influence contributed to this variability, modifying the trophic structure of the upper water layers. The particulate organic carbon (POC) fraction potentially respired per day by ETS activity was approximately 2.1-2.6 % in the 0-50 m layers, increasing to 3.8-5.7% in the 100-200 m-deep layer. The ROME 2 deep layer showed a significantly lower potential respiration of POC than the other areas and few significant correlations with POM quantity and quality features. In the other areas, instead, significant correlations were common, especially in ROME 3, indicating that in the offshore sites the POM was the main organic fuel of respiration.

Keywords: Microbial respiration; Particulate organic matter; Trophic regimes; Ross Sea

1. Introduction

The flux of particulate organic matter (POM) from the surface of the ocean to the bottom depends on the physical features of the water column (e.g. stratification, hydrological structures, turbulence, light penetration), the chemical properties of the POM itself and the prevailing planktonic food chain (type of phytoplankton and zooplankton, microbial loop, etc) (Wassman et al., 1994). The role of this flux within the ecological processes of the sub-surface water layers is tightly linked to the ability of organisms to oxidise the POM to gather energy and materials. The amount, distribution and variability of respiration rates in the marine environments constitutes an index of the organic matter oxidation (La Ferla et al., 1996, 1999). Among the different approaches to evaluate respiratory activity, the Electron Transport System activity (ETSa) provides a sensitive proxy of oxygen consumption rates. Moreover, it estimates the oxidation of both POM and dissolved organic matter (DOM). As a consequence, the use of the assay for ETSa has gained acceptance in aquatic ecology (Williams and Del Giorgio, 2005; La Ferla et al., 2010).

It has been demonstrated that respiration can exceed photosynthesis in large areas of the ocean (Duarte et al., 1999; Williams, 1998). In addition, the higher constancy of the rates of microbial respiration than those of photosynthetic production has been a major assumption in oceanography (Karl et al., 2003). Oceanic microbial respiration is considered less variable than photosynthesis because heterotrophic microbes forage on the entire organic matter (OM) pool, that is quantitatively more stable and larger than that just derived from local primary production (Karl et al., 1998). Short and intense bursts of photosynthesis can explain this observation. They occasionally charge the organic reservoir, while respiration slowly and steadily discharges it (Karl et al., 2003). Mesoscale phenomena are mechanisms that could generate high-frequency increases of photosynthesis, supporting a higher and long-term heterotrophic reworking and respiration of organic debris (Gonzalez et al., 2001; Maixandeau et al., 2005). Enhanced respiration rates have been associated with anticyclonic eddies in the Canary Islands region (Arístegui and Montero, 2005) and Mediterranean eddies in the Atlantic Ocean, close to Gibraltar Strait (Savenkoff et al., 1993). Moreover, an important variability in gross photosynthesis and respiration rates was reported associated with three mesoscale eddies in the Sargasso Sea (Mourino-Carballido and McGillicuddy, 2006) and in North Western subtropical Atlantic (Mouriño-Carballido, 2009). The information provided for polar regions, such as Antarctica, (Estrada et al., 1992; Crisafi et al., 2000; Ducklow et al., 2000; Arístegui et al., 2002; Azzaro et al., 2006; 2007; Catalano et al., 2010) is rather low and characterised by a limited spatial or temporal scale of investigation.

In order to fill this gap, we present here data on the microbial respiration rates and their relationships with POM amounts and biochemical composition, recorded in three different areas of Ross Sea during summer 2014 (Cruise ROME-2014). The objectives of this study are: i) to measure the range of the respiratory ETSa values and the POM fraction potentially respired by the microbial component; ii) to highlight the influence of the POM features and of the environmental constraints on the ETSa.

2. Material and Methods

2.1 Study area and sampling

The sampling was performed on board of the R/V Italica in three different areas of the Ross Sea (Fig. 1). A detailed analysis of the physical features of the three studied areas has been provided by Rivaro et al. (this issue) and Misic et al. (submitted). The ROME 1 stations (located at approximately 170°E and 75°S) were separated by a frontal area: stations 9 and 13 were characterised by a higher salinity, temperature and depth of the upper mixed layer (UML, higher than 30 m). The others, especially the station 20, were subjected to the influence of residual ice, with the UML shallower than 15 m. The ROME 2 area, placed coastward and next to the winter Terra Nova Bay polynya, showed a front involving stations 34 and 45, where the water masses converged. Local features produced a rather thin UML (shallower than 15 m) in the western stations (36 and 43), while the eastern station 39 showed a deeper UML (24 m). Finally, the ROME 3 area, sited in the southern Ross Sea at 168°E towards the Ross Ice Shelf, showed a cyclonic eddy that involved the main part of the stations, except the 48 and the 52, the former showing opposite current direction and the latter higher salinity values and the deepest UML (75 m). On average, the mixed layer of ROME 3 (51±31 m) was significantly (one way ANOVA, $p<0.05$) deeper than ROME 1 (25±12 m) and ROME 2 (16±5 m).

Fluorescence profiles were acquired by means of a SBE 9/11 Plus probe mounted on a rosette sampler. The sampler was equipped with 12-L Niskin bottles, used to perform the samplings for ETSa and POM at 17 stations (Fig. 1). Samples were collected in the epipelagic layer at 4 fixed depths (surface, 50, 100 and 200 m) and 1 variable depth, depending on the maximum of the signal for fluorescence. Additional samplings, focused on the ETSa analysis, were performed in several stations within the 0-200 m layer (generally, 70-80 m for ROME 1 and ROME 2, 30-35 m for ROME 3) and in the mesopelagic layer (455±55 m, 751±56 and 700±43 for ROME 1, ROME 2 and ROME 3, respectively).

2.2 Electron Transport System activity (ETSa)

Respiratory rates were obtained according to the tetrazolium reduction technique (Packard, 1971, 1985), as modified by Kenner and Ahmed (1975) for the microplankton community. The ETSa assay allows an estimate of the maximum velocity (Vmax) of the dehydrogenases transferring electrons from their physiological substrates (NADH, NADPH, and succinate) to a terminal electron acceptor (O_2) through their associated electron transfer system. Briefly, subsamples for the analysis were prefiltered through a 250-mm mesh size net to remove large particles and concentrated on GFF-glass-fibre filters (nominal pore diameter 0.7 µm) at reduced pressure (<1/3 atm). Although the filter porosity was specific for microplankton, GFF filters retain also particles colonised by very small heterotrops. The filters were folded into cryovials and immediately stored in liquid nitrogen until they were analysed in the laboratory (<45 days) to prevent enzymatic decay (Ahmed et al., 1976). The ETSa was corrected for in situ temperature with the Arrhenius equation using a value for the activation energy of 11.0 kcal/mol (Azzaro et al., 2006). The ETSa conversion to carbon and day units was performed according to Azzaro et al. (2012).

2.3 Particulate Organic Matter (POM)

Seawater was filtered through GFF-glass-fibre filters. Water volume ranged from 0.5 L (surface and maximum of fluorescence) to 1 L (deeper depths). Analysis were carried out in duplicate.

Particulate organic carbon (POC) was analysed following Hedges and Stern (1984), after acidification with HCl fumes in order to remove inorganic carbon. The cyclohexanone 2-4-dinitrophenyl hydrazone was used to calibrate a Carlo Erba Mod. 1110 CHN Elemental Analyser. The specific standard deviations, due to the analytical procedures and sample handling, was 7.4%.

Particulate proteins and particulate carbohydrates were analysed following Hartree (1972) and Dubois et al. (1956), respectively. Albumin and glucose solutions were used to calibrate a Jasco V530 spectrophotometer. The specific standard deviations were 8.3% and 15.5% for proteins and carbohydrates, respectively.

2.4 Statistical analysis

We tested the differences of the same variable between different samplings with the one-way ANOVA. This test was followed by the Newman-Kneuls post-hoc test (ANOVA-NK test) (Statistica software). To test the relationships between the various parameters, a Spearman-rank correlation analysis was performed. The Principal Component Analysis (PCA) was applied on the normalised data of POC, protein and carbohydrate concentrations and of the protein/carbohydrate ratio (PRIMER software). The observations were statistically clustered (cluster analysis performed on the normalised data, resemblance measure: Euclidean distances, cluster mode: group average), and the similarity percentage analysis (SIMPER) was used to highlight the parameters responsible of such groupings.

3. Results

The presentation of the ETSa and the POM data is performed according to the physical features previously described. The data were averaged for each depth, merging the stations that showed peculiar characteristics for each area. Therefore, the trends with depth of "ROME 1 ice" were related to the stations (16 and 20) that showed a certain influence of a longer ice presence. "ROME 2 front" was the average of stations 34 and 45, involved in the convergence of the water masses neighbouring the front. "ROME 3 eddy" derived from the eddy-involved stations (50, 55, 56, 67, 69 and 75). The remaining stations constituted the basic conditions for each area: ROME 1 (stations 9 and 13), ROME 2 (36, 39 and 43), ROME 3 (48 and 52).

Fig. 2 (A, B and C) reports the vertical distribution of ETSa (in µl O_2 l^{-1} h^{-1}) in the selected sites. In general, ETSa decreased with depth. Averaging the water columns down to 700 m, all the areas showed that the 50% of the total ETSa was expressed within the 0-50 m layer, and at 100 m the 70-80% was reached. The differences between the ETSa of the three sites were quite large. ETSa measurements ranged from 0.09 to 0.36 µl O_2 l^{-1} h^{-1} in ROME 1, between 0.10 and 1.18 µl O_2 l^{-1} h^{-1} in ROME 2 and from 0.05 to 1.07 µl O_2 l^{-1} h^{-1} in ROME 3. The lowest values were recorded in ROME 1 (along the entire water column, $p<0.01$) and the highest in ROME 3 (first 20 meters, $p<0.01$). In the entire epipelagic layer (0-200 m), the lowest and highest mean ETSa values were measured in ROME 1 (0.20±0.02 µl O_2 l^{-1} h^{-1}) and ROME 2 (0.49±0.19 µl O_2 l^{-1} h^{-1}), respectively. An intermediate mean value was registered in ROME 3 (0.34±0.17 µl O_2 l^{-1} h^{-1}). A similar result, but with lower values, was found in the mesopelagic layer ($200<z<700$ m - ROME 1, 0.11±0.02 µl O_2 l^{-1} h^{-1}; ROME 2, 0.20±0.08 µl O_2 l^{-1} h^{-1}; ROME 3, 0.14±0.05 µl O_2 l^{-1} h^{-1}). Generally, the hydrological structures identified in three areas did not affect the levels of respiratory activity. Only the front-influenced stations of

ROME 2 showed significantly higher ETSa values than the other stations at 25 and 100 m ($p<0.05$). Calculating the ETSa converted to carbon and day units, the averaged values were 2.0±0.7, 3.7±2.5 and 3.6±2.3 μg C l^{-1} d^{-1} for ROME 1, ROME 2 and ROME 3, respectively.

Fig. 3 reports the trends with depth of POC (A, B and C), protein (D, E and F) and carbohydrate (G, H and I) concentrations. The ROME 1 normal and ice-influenced stations and the ROME 3 normal and eddy-influenced stations showed rather sharp decreasing trends with depth of the concentrations of all the considered variables. The ice-influenced stations of ROME 1 showed lower values than the other stations of the same area, especially in the 0-50 m layer. The eddy-influenced stations of ROME 3, instead, showed higher values than the normal stations, not directly influenced by this physical constraint. No differences were found between the ROME 2 normal and front-related stations, showing rather high values in the entire water column. The carbohydrates were an exception, showing higher values for the frontal stations than for the others.

The POC/fluorescence ratio values (Fig. 4 A-C) highlighted the contribution of the phytoplankton to the POM bulk, knowing that the lowest the ratio the highest the phytoplanktonic contribution to the organic particulate. A higher phytoplanktonic contribution was observed for ROME 1 ice-influenced stations and for the 0-20 m layer of ROME 3 eddy-influenced stations than the respective normal station trends. No difference was observed, instead, for ROME 2 normal and front-influenced stations. Anyway, all the sampled depths of ROME 2, except the surface one, showed rather low ratio values.

The POC fraction potentially respired by ETSa per day was higher for the deeper sampled layers than for the surface and subsurface ones. ROME 1 and ROME 2 showed, on average for the 0-50 m deep layer, similar values of 2.1±1.7% and 2.1±1.5%. They were slightly lower than those calculated for ROME 3 (2.6±1.4%). In the 100-200 m layer ROME 2 showed the lowest ($p<0.05$) respired POC fraction (3.8±1.5%), while ROME 1 and ROME 3 showed values of 5.7±2.4% and 5.6±1.8%, respectively.

Besides the quantitative information given by the single concentrations, the protein/carbohydrate ratio (Misic and Fabiano, 1996) gave clues on the qualitative value of particulate for consumer. Proteins are known to be biologically available (Etcheber et al., 1999), while the carbohydrates we measured (cellulose, for instance) need energy-expensive hydrolytic activity to be degraded (Misic and Covazzi Harriague, 2008). Therefore, the highest the protein/carbohydrate ratio, the highest the trophic value of POM. The protein/carbohydrate ratio (Fig. 4 D-F) indicated a lowering of the POM trophic quality for the stations influenced by ice, front and eddy, with the lowest values observed at ROME 2 ice-influenced stations

(1.6±0.4). ROME 2 showed the lowest mean values also considering all the stations and depths (2.0±0.8 vs 2.2±1.0 and 3.6±1.0 for the entire ROME 1 and the entire ROME 3, respectively).

The PCA (Fig. 5A) was based on the POM values, i.e. the analysis was limited to the epipelagic layer. PC1 explained 72.5% of the variability, PC2 25.2%. This multivariate analysis confirmed the differences between the main part (94%) of the deeper observations (100-200 m deep, indicated in the figure as DL), characterised by lower values, and the main part (87%) of the surface and sub-surface values (0-50 m deep, indicated as SL). Some 100-m-deep observations, belonging to the front-influenced stations of ROME 2, showed a higher similarity with the shallower depths. The SIMPER analysis applied on the clusters highlighted that the division within the 0-50 m layer observations (indicated as SLa, dominated by the ROME 3 observations -57% - and SLb, dominated by the ROME 2 observations – 52%) were driven by the protein/carbohydrate ratios (38%) and carbohydrate concentrations (32%) (Table 1).

Superimposing the ETSa values on the PCA plot (Fig. 5B), generally the highest values characterised the observations with the highest protein and protein/carbohydrate ratio values (cluster SLa). Nevertheless, very high ETSa values were recorded for some depths of ROME 2, that showed the lowest protein/carbohydrate ratio, suggesting that POM trophic quality shaped differently the microbial respiration depending on the considered area. This fact was confirmed by the correlations between ETSa and the POM variables reported in Table 2. While ROME 1 and ROME 3 showed positive and significant relationships between ETSa and nearly all the variables, ROME 2 showed very few correlations and, in particular, the absence of correlation with the trophic quality of POM (protein/carbohydrate ratio).

The ratio between the ETSa and the primary biomass (ETSa/chlorophyll-a ratio, Martinez, 1991) reflects the potential contribution of autothrophs to the total microplankton respiration. The ETSa in the ice-, front- and eddy-influenced stations showed a higher phytoplanktonic contribution (fluorescence/ETSa ratio) than the other stations (Fig. 6A-C), the highest at ROME2 between 20 and 50 m.

The ratio between ETSa and organic substrates, POC for instance, has been used as an index of POC quality and degradability (Relexans et al.,1992). The front-influenced stations of ROME 2 showed the highest values of the ETSa/POC ratio (Fig. 6 D-F), i.e. the lowest trophic quality at the same depths where the phytoplanktonic contribution was the highest. Similarly, also ROME 3 showed a lower trophic value, where the autotrophic contribution was the highest.

4. Discussion

Our knowledge on microbial oxidation of organic matter (OM) in polar regions is still rather poor and little information is available regarding the response of the respiration activity to the OM features. In our study we found that, irrespectively of the low temperatures, the ETSa was consistent with observations from temperate and tropical areas (Arístegui et al., 2003; Arístegui and Montero, 2005; Baltar et al., 2010) and they were within the ranges reported by Martinez and Estrada (1992), Arístegui et al. (2002) and Catalano et al. (2010) for the Antarctic areas.

The different position of the study areas and the presence of peculiar hydrological features shaped the trophic structure of the upper water layers, characterised by the variability of POM quantity and quality, that influenced the oxidation rates (Relexans et al., 1992; Martinez, 1997; Baltar et al., 2015). Significant differences were, in fact, observed within the three areas, according to previous investigations. Azzaro et al. (2006) observed a quantitative difference of ETSa in the 100-1000 m layer of stations placed approximately where the ROME 1 and ROME 3 were located, with higher values in the southernmost area during summer 2001.

Surprisingly, the stations characterised by the presence of peculiar hydrodynamic features (in particular the front of ROME 1 and the eddy of ROME 3) showed no significant differences for the oxidation activity when compared to the respective normal stations. Each macro-area expressed a rather constant mean oxidation potential, perhaps the maximum expressible by the resident microbial communities. We hypothesise that the ETSa was mainly regulated by the differences in the composition of the microbial fraction, according to the spatial and seasonal changes (Smith and Asper, 2001; Abell and Bowman, 2005). These observations are supported by those studies that pointed to a rather low variability of microbial respiration, due to the ability of microorganisms to slowly and steadily discharge the OM reservoir (Karl et al., 2003; Robinson, 2008).

The ETSa and POM variations with the geographical position led to variable POM fractions potentially respired. The higher values of the deeper layers pointed to a diffused and notable recycling activity by the heterotrophic microbes. The POC fraction potentially respired by ETSa was, on average, similar to that reported by Martinez (1991) for the Barents Sea (3-4%), although the ETSa converted to carbon and day units showed lower values than those recorded in the Arctic area (5 µg C l^{-1} d^{-1}). This result indicates the need to better understand the relationship between ETSa and POM, that has been indicated as the main trophic target for the microbial heterotrophs (Arístegui et al., 2003; Azzaro et al., 2006).

ROME 1 was a rather large area, but the environmental features led to similarly low microbial ETSa. The western sites (ice-influenced ROME 1 stations) showed the features of initial phytoplanktonic production and low POM concentrations of rather low food quality. Microbial ETSa was significantly correlated to quantitative as well as qualitative POM features, confirming its dependence on the occurrence of available/labile organic matter (Relexans et al., 1992). But in these stations ETSa was not correlated to fluorescence, indicating that the heterotrophic microbial fraction was still predominant (Martinez and Estrada, 1992). In the other stations of ROME 1 the correlation with fluorescence was significant, but microbial ETSa was similarly low. In this case a more complex trophic web, with a higher activity of the mesoplanktonic fraction, was probably established, due to the stabilised ice-free conditions. Tagliabue and Arrigo (2003), applying an ecological model, showed that the highest abundances of mesozooplankton in the Ross Sea summer polynya occurred from late December to late January (when the sampling of ROME 1 took place), while in other areas (Terra Nova Bay polynya, for instance) the mesozooplankton peaked later. The ROME 1 the mesozooplankton had, likely, influenced the microbial component with a top-down limitation, and had contributed to the respiration of POM. Therefore, the oxidation of the POM was a matter also of the higher dimensional fractions of the heterotrophic organisms, that were not measured.

Moving southward, towards the Ross Ice Shelf, the ROME 3 area was characterised by the presence of a cyclonic eddy, that interested the main part of the sampled stations. In the ROME 3 area, the ETSa was higher than in the other two study areas especially in the surface and subsurface layers. Previous studies reported the variability of the respiration rates associated with different types of hydrodynamic mesoscale features. Enhanced respiration and activity rates have been associated with mesoscale eddies in the Canary Islands region (Arístegui and Montero, 2005; Baltar et al., 2010). Maixandeau et al. (2005) reported that spring stratification disrupted the mesoscale eddy structure and reduced the positive influence of the eddy on respiration rates. Therefore, the rather deep mixed layer of ROME 3 may have favoured the positive eddy influence on the OM production and oxidation rates. A direct and indirect enhancement of ETSa has been observed in turbulent conditions, either in the natural environment (Arístegui and Montero, 2005) and in laboratory experiments (Peters and Marrasé, 2000, and references therein). The high current velocities recorded in the ROME 3 area (Misic et al., submitted) could, then, be co-responsible of the increased activities recorded. The hydrodynamic forcing stimulated the phytoplanktonic activity, with biomass accumulation especially in the 0-20 surface layer, as shown by the POC/fluorescence ratio values. The good

trophic quality of POM confirmed the presence of freshly produced (phytoplanktonic) POM, ready to be respired. The 0-50 m deep layer of these stations (namely the group SLa in Fig. 5A) showed the highest protein/carbohydrate ratios (SIMPER analysis). The ETSa may also be an indicator of biomass (Packard, 1985; Relexans et al., 1992). Therefore, besides a rather high phytoplanktonic contribution, the metabolism increase pointed to a richer heterotrophic assemblage.

The PCA pointed to clear vertical differences of the POM distribution, highlighting general POM depleted conditions for the 200 m deep observations, that were significantly lower than the respective upper water layers. ROME 1 and ROME 3 showed generally depleted conditions also at the 100 m deep layer, confining the main POM accumulation and turnover in the upper 50 m layer. On the other hand, the peculiar physical features of ROME 2 caused a rather unchanged POM concentration along the water column down to 100 m depth. This was particularly true for the front-influenced stations, that experienced a convergence of the water masses and a deepening of the primary biomass signal. But, by a qualitative point of view, a roughly constant vertical distribution of the POM was not necessarily a favourable feature for consumers. The lower protein/carbohydrate ratio values of ROME 2 pointed to the presence of a more refractory trophic supply, needing a higher energy devoted to its utilisation (as shown by rather high POM/ETSa ratio values).

Previous studies on the heterotrophic microbial community of the Terra Nova Bay (TNB), approximately where ROME 2 was placed, were focused on bacterioplankton and highlighted significant differences of abundance and activity with offshore areas such as Cape Adare (Povero et al., 2006; Celussi et al., 2009). Higher abundances during summer have been recorded for TBN, coupled with lower hydrolytic activities, especially those related to complex and refractory glucidic compounds such as cellulose (β-glucosidase activity, for instance). Povero et al. (2006) indicated in the TNB area a lower trophic quality of POM, suggesting limiting trophic conditions that would explain the average prokaryote carbon production found by Celussi et al. (2009). Our ETSa evaluation adds new insights into this debate. ETSa values of the ROME 2 stations, placed next to the TNB polynya, were intriguingly scarcely correlated to the POM quantity and not correlated to the POM quality. A high variability of ETSa was recorded, with significantly higher ETSa values at either 25- or 100-m-deep layers of the front-influenced stations. In these stations dissolved OM and POM accumulated, due to the water convergence (Arístegui and Montero, 2005), while at the other stations the anomalous higher values were confined in the 40-50 m layer. Increases of ETSa values in frontal areas have been observed previously in other environments. Arìstegui et al. (2002) and Azzaro et al. (2007)

found higher values at the Antarctic Polar Front than in the neighbouring oceanic sites. In the Mediterranean Sea (North Adriatic Sea), La Ferla and Azzaro (2001) and La Ferla et al. (2006) observed that OM oxidation was 2.7 times higher inside a frontal system, caused by strong riverine discharge, than outside the front.

A fraction of the ETSa may be due to autotrophs (Martinez and Estrada, 1992; Chapman et al, 1994; Arístegui and Montero, 1995), as indicated by the values of the POC/fluorescence ratio and of the fluorescence/ETSa ratio at ROME 2. The presence of autotrophic biomass would led to overestimation of the heterotrophic oxidation processes. Nevertheless, no significant correlation was found between ETSa and fluorescence at ROME 2. The highest ETSa values were related to the highest phytoplanktonic contribution only two times out of five, suggesting that they should be related also to other constraints. The PCA analysis, unexpectedly, pointed to the low protein/carbohydrate ratio values (SIMPER). This was a contradiction, but probably only apparent. In trophically limited environments, in fact, the organisms that maintain high enzymatic expression, although not active, could have an advantage in case of abrupt increases of the trophic supply (Packard et al., 1996). Another hypothesis to explain the anomalous ETSa was related to the effort that the microbial consumers would take to adapt to unfavourable trophic conditions. The highest particulate carbohydrate concentrations need the energy-consuming synthesis of peculiar hydrolytic enzymes (namely the generally poorly expressed β-glucosidase and the other hydrolytic enzymes related to the cellulose lysis), thus increasing the ETSa in terms of energy provider to perform those biochemical processes. This fact would be also the reason of the significant correlation between carbohydrates and ETSa. The gain in this process may, actually, be of some use for consumers. Previous studies carried out on the growth rates of microorganisms of Antarctic soils (Bolter, 1993) showed clearly the preference of the microbial community (especially bacteria) for mono- and disaccharides and polymers as starch, characterised by rather easy cleavage, emphasizing their importance for microbial metabolism. This preference for carbohydrates confirms earlier findings, that showed that glucose is respired and incorporated rapidly into the biomass when added to a soil solution (Roser et al., 1993).

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Captions to Figures

Figure 1. Map of the stations of the ROME 1, ROME 2 and ROME 3 areas.

Figure 2. ETS activity scatterplots versus depth (epipelagic and mesopelagic layers) at the three sites sampled in the Ross Sea. The ETS activity has been averaged for each depth for the stations that showed peculiar hydrodynamic features (dotted lines for ice-influenced stations of ROME 1, for front-influenced stations of ROME 2, for eddy-influenced stations of ROME 3) and normal hydrodynamic features (continuous line). See text for details.

Figure 3. Vertical trends of POC, protein and carbohydrate concentrations in the three sampled areas for the epipelagic layer. Continuous and dotted lines as in Figure 2 and text.

Figure 4. Vertical trends of phytoplanktonic biomass contribution to the POM bulk (POC/fluorescence ratio) and of the trophic quality or POM (protein/carbohydrate ratio) in the three sampled areas for the epipelagic layer. Continuous and dotted lines as in Figure 2 and text.

Figure 5. PCA for the POM variables of the epipelagic layer. A: markers point to the depth of the observations. Cluster analysis is superimposed (dotted ellipses), highlighting two main clusters: SL and DL. SLa and SLb represent a further grouping inside the cluster SL (see text for details). B: ETS activity values are superimposed on the previous PCA. Yellow stars highlight the five anomalous ETSa values of ROME 2 (see text).

Figure 6. Vertical trends of phytoplanktonic contribution to the ETSa (fluorescence/ETSa ratio) and of the trophic quality or POM (POC/ETSa) in the three sampled areas for the epipelagic layer. Continuous and dotted lines as in Figure 2 and text.

Table(s)

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Table 1. SIMPER analysis on the entire POM data set, grouped by cluster analysis as presented in Figure 5.

cluster	variable	contribution %
SL vs DL	proteins	27
	POC	27
	carbohydrates	25
	proteins/carbohydrates	21
SLa vs SLb	proteins/carbohydrates	38
	carbohydrates	32
	POC	15
	proteins	15

Table 2. Significant correlations between ETSa and POM variables. The first coefficients are given for each of the three areas (ROME 1, ROME 2 and ROME 3). Below, the coefficients are related to the stations that, in each area, showed similar physical properties (see text for details).
***: p<0.001, **: p<0.01, *: p<0.5.

	n	POC	protein	carbohydrate	protein/ carbohydrate	fluorescence
ROME 1	17	0.74***	0.77***	0.70**	0.84***	0.57*
ROME 2	22	0.44*		0.47*		
ROME 3	37	0.78***	0.79***	0.63***	0.57***	0.69***
ROME 1	9	0.73*	0.76*		0.93	0.72*
ROME 1 ice	8	0.75*	0.78*	0.72*	0.80*	
ROME 2	13			0.78**		0.56*
ROME 2 front	9					
ROME 3	9	0.95***	0.93***	0.86**	0.74*	0.86**
ROME 3 eddy	28	0.74***	0.77***	0.61***	0.54**	0.68***

Figure 1

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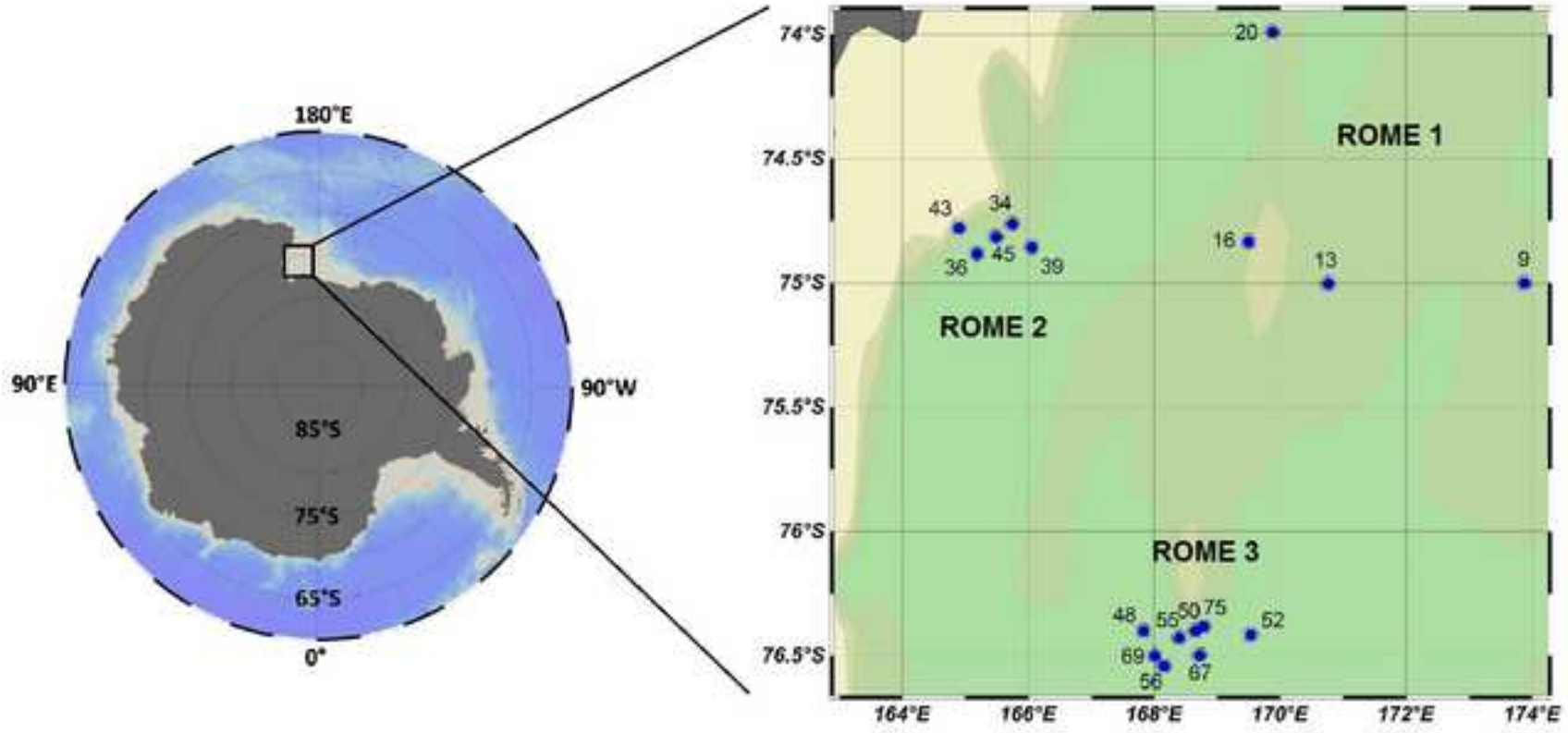

Figure 2

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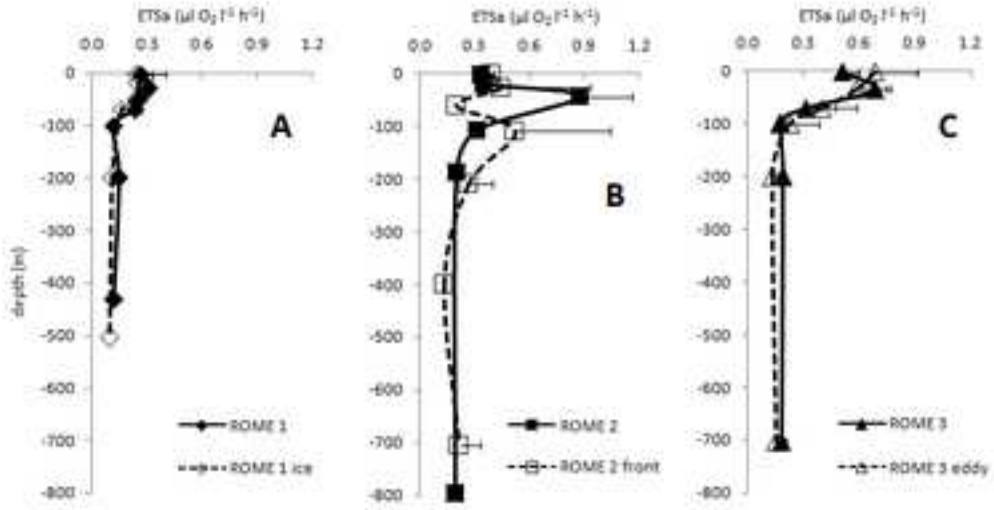

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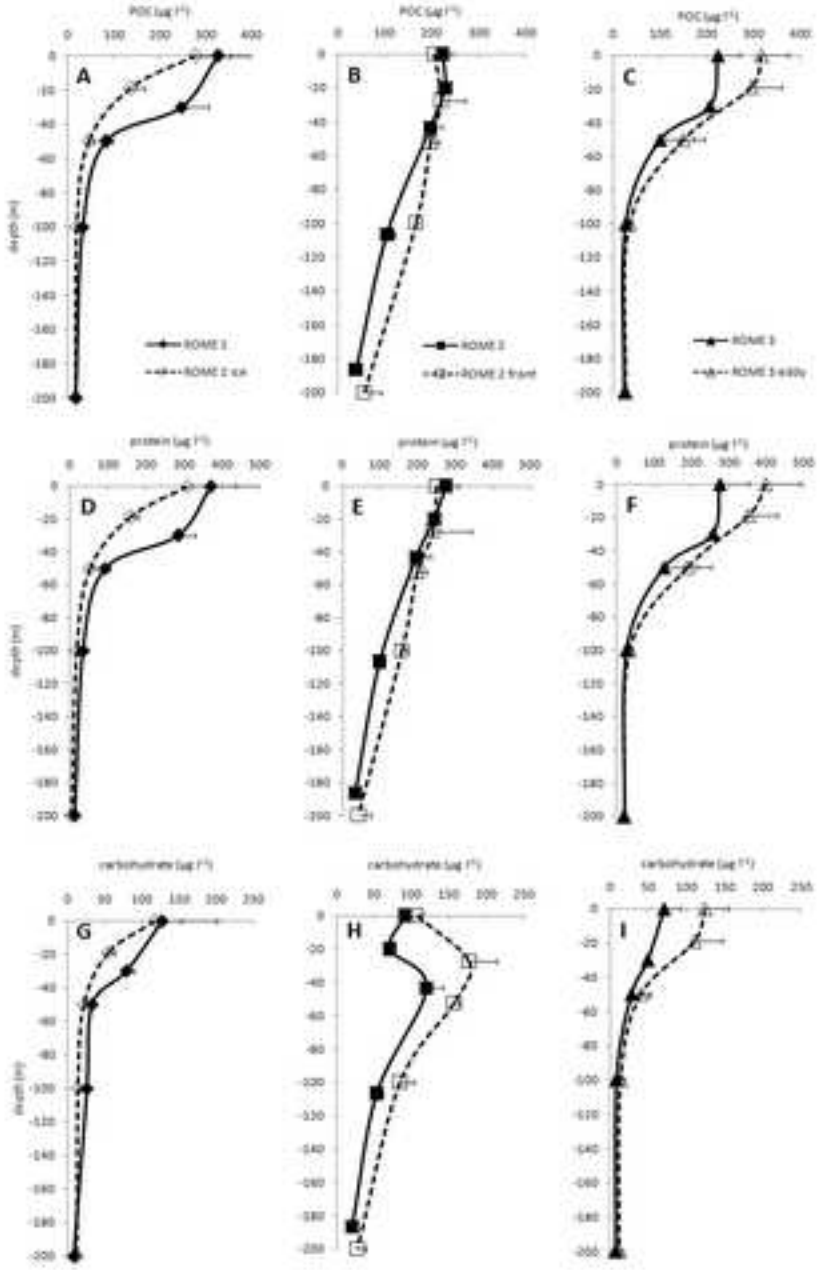

Figure 4

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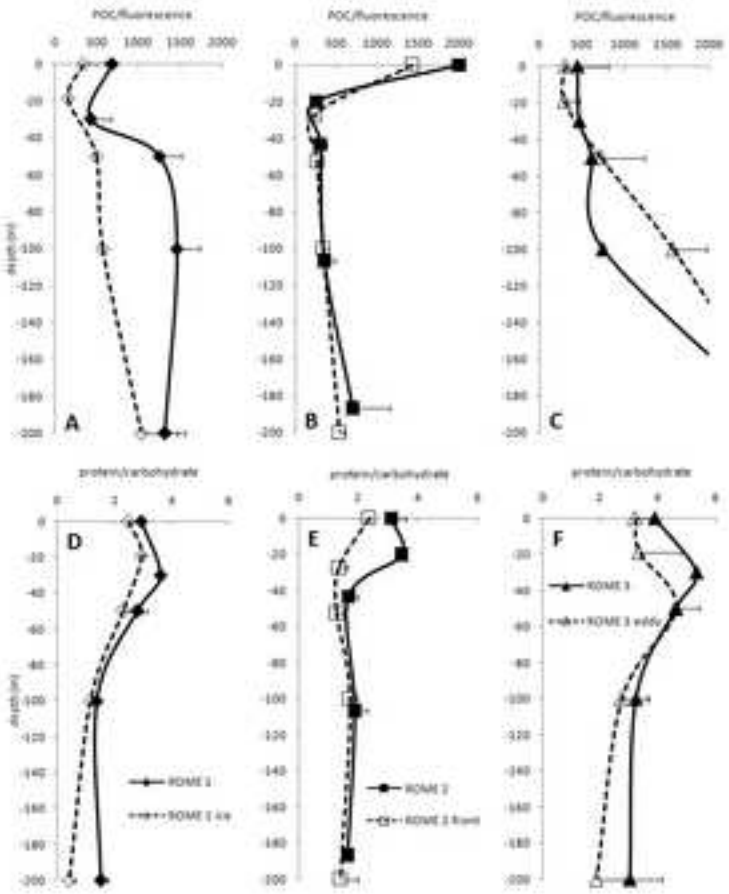

Figure 5

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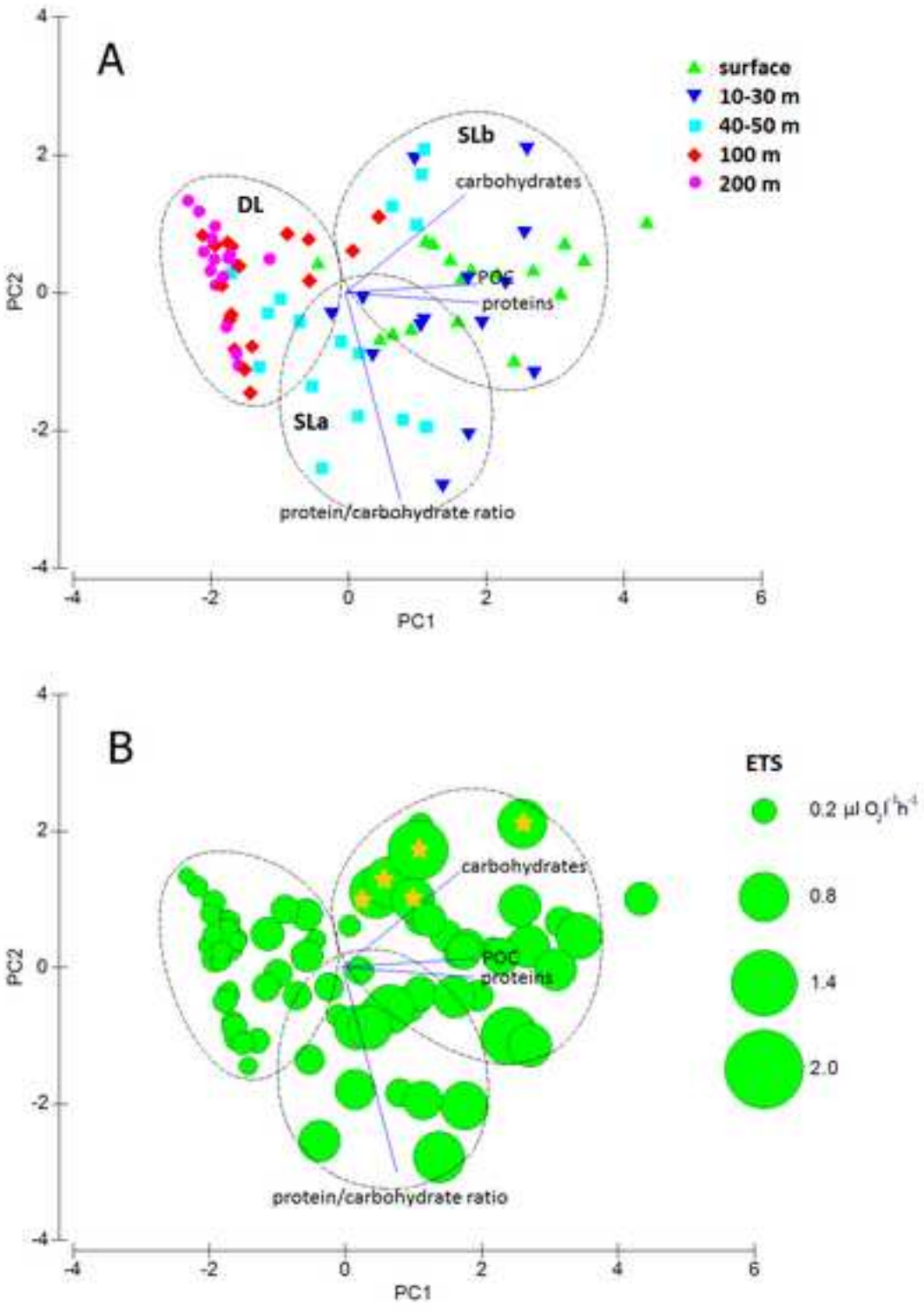

Figure 6

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